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(54) Title: A NOVEL DISINTEGRIN METALLOPROTEASE AND METHODS OF USE

(57) Abstract

This invention provides a method for identifying compounds capable of binding to the disintegrin protein, and determining the amount and affinity of a compound capable of binding to the disintegrin protein in a sample. This invention also provides a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein and the human disintegrin metalloprotease protein, fragment or mutant thereof, useful for these purposes. This invention also provides an *in vivo* or *in vitro* method for screening for osteoarthritis and other metalloprotease based diseases, capable of manufacture and use in a kit form.

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A NOVEL DISINTEGRIN METALLOPROTEASE AND METHODS OF USE

Field of the invention

The invention relates to a novel protein, its fragments and mutants and to its use in detecting and testing drugs for ailments, including osteoarthritis and others characterized by up regulation of metalloproteases.

Background

A number of enzymes effect the breakdown of structural proteins and are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metalloprotease enzymes, as are the angiotensin-converting enzymes and the enkephalinases. Collagenase, stromelysin and related enzymes are important in mediating the symptomatology of a number of diseases, including rheumatoid arthritis (Mullins, D. E., et al., Biochim Biophys Acta (1983) 695:117-214); osteoarthritis (Henderson, B., et al., Drugs of the Future (1990) 15:495-508); the metastasis of tumor cells (ibid, Broadhurst, M. J., et al., European Patent Application 276,436 (published 1987), Reich, R., et al., 48 Cancer Res 3307-3312 (1988); and various ulcerated conditions. Ulcerative conditions can result in the cornea as the result of alkali burns or as a result of infection by Pseudomonas aerugin sa, Acanthamoeba, Herpes simplex and vaccinia viruses.

Other conditions characterized by undesired metalloprotease activity include periodontal disease, epidermolysis bullosa and scleritis. In view of the involvement of metalloproteases in a number of disease conditions, attempts have been made to prepare inhibitors to these enzymes. A number of such inhibitors are disclosed in the literature. The invention seeks to provide novel inhibitors, preferably specific to this protease, that have enhanced activity in treating diseases mediated or modulated by this protease.

Inhibitors of metalloproteases are useful in treating diseases caused, at least in part, by breakdown of structural proteins. A variety of inhibitors have been prepared, but there is a continuing need for metalloprotease inhibitor screens to design drugs for treating such diseases.

Metalloproteases are a broad class of proteins which have widely varied functions. Disintegrins are zinc metalloproteases, abundant in snake venom. Alternate cloning strategies could be used. Mammalian disintegrins are a family of proteins with about 18

known subgroups. They act as cell adhesion disrupters and are also known to be active in reproduction (for example, in fertilization of the egg by the sperm, including fusion thereof, and in sperm maturation).

These proteases and many others are uncovered in molecular biology and biochemistry. As a result, Genbank, a repository for gene sequences, provides several sequences of metalloproteases, including some said to encode fragments of disintegrins. For example, GenBank accession # Z48444 dated February 25, 1994 discloses 2407 bases of a rat gene said to be a rat disintegrin metalloprotease gene; GenBank accession # Z48579 dated March 2, 1995 discloses 1824 bases of a partial sequence of a gene said to be a human disintegrin metalloprotease gene; GenBank accession # Z21961 dated October 25, 1994, discloses 2397 bases of a partial sequence of a gene said to be a bovine zinc metalloprotease gene.

It would be advantageous to implicate metalloproteases in specific disease states, and to use these metalloproteases as tools to detect and ultimately cure, control or design cures for such diseases.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide a method for identifying compounds capable of binding to the disintegrin protein.

It is also an object of the present invention to provide a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein.

It is also an object of the present invention to provide a method for screening for metalloprotease mediated diseases such as cancer, arthropothies (including ankylosing spondolytis, rheumatiod arthritis, gouty arthritis (gout), inflammatory arthritis, Lyme disease and osteoarthrtis).

It is also an object of the present invention to provide an antibody to the protein useful in the screen, in the isolation of the protein or as a targeting moiety for the protein.

SUMMARY OF THE INVENTION

This invention provides a method for identifying compounds capable of binding to the disintegrin protein, and determining the amount and affinity of a compound capable of binding to the disintegrin protein in a sample.

This invention also provides a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein and the human disintegrin metalloprotease protein, fragment or mutant thereof, useful for these purposes.

This invention also provides an in vivo or in vitro method for screening for osteoarthrtis and other metalloprotease based diseases, such as cancer, capable of manufacture and use in a kit form.

DETAILED DESCRIPTION

As used herein, the terms "protein," "protease," and "metalloprotease" refer to a disintegrin. Preferably this is a human disintegrin as described below.

The term "antibody" refers to an antibody to a disintegrin, or fragment thereof. These many be monoclonal or polyclonal, and can be from any of several sources. The invention also contemplates fragments of these antibodies made by any method in the protein or peptide art.

The term "disease screen" refers to a screen for a disease or disease state. A disease state is the physiological or cellular or biochemical manifestation of the disease. Preferably this screen is used on body tissues or fluids of an animal or cell culture, using standard techniques, such as ELISA. It also contemplates "mapping" of disease in a whole body, such as by labeled antibody as described above given systemically: regardless of the detection method, preferable such detection methods include fluorescence, X-ray (including CAT scan), NMR (Including MRI), and the like.

The term "compound screen" is related to the methods and screens related to finding compounds, determining their affinity for the protease, or designing or selecting compounds based on the screen. In another embodiment, it contemplates the use of the three dimensional structure for drug design, preferable "rational drug design", as understood by the art. It may be preferred that the protease is in "essentially pure form", which refers to a protein reasonably free of other impurities, so as to make it useful for experiments or characterization. Use of this screening method assists the skilled artisan in finding novel structures, whether made by the chemist or by nature, which bind to and preferably inhibit the protease. These "inhibitors" may be useful in regulating or modulating the activity of the protease, and may be used to thus modulate the biological cascade that they function in. This approach affords new pharmaceutically useful compounds.

The term "disintegrin" refers to a disintegrin, a fragment thereof, a mutant thereof or a homologue which still retains its function. This term contemplates aggracanase, and other proteases which are involved in or modulate tissue remodelling. This contemplates disintegrins from differing species, and those prepared by recombinant methods, in vitro methods, or standard peptide synthesis. Preferably the protein is a human disintegrin or mutant thereof. For the purposes of defining the mutants of the protein the preferred

"native" protein is described in GenBank accession # Z48579, incorporated herein by reference and referred to in the sequence below. SEQ ID NO 1 describes a fragment of that DNA sequence and its transcript and SEQ ID NO 2 describes the coding region of the gene and its transcript. Homologue disintegrins include whole proteins with af least 90% homology as understood by the art, or fragments thereof. For example, a rat protein which is 95% homologous to that of SEQ ID NO. 1 or SEQ ID NO. 2 based on the peptide sequence derived from the DNA or cDNA sequence, and a bovine protein (similarly derived) being 97-98% homologous, are both considered homologues. Thus homologous cDNAs cloned from other organisms give rise to homologous proteins.

Likewise proteins may be considered homologues based on the amino acid sequence alone. Practical limitations of amino acid sequencing would allow one to determine that a protein is homologous to another using for example comparison of the first 50 amino acids of the protein. Hence 90% homology in would allow for 5 differing amino acids in the chain of the first 50 amino acids of the homologous protein.

The skilled artisan will appreciate that the degeneracy of the genetic code provides for differing DNA sequences to provide the same transcript, and thus the same peptide. In certain cases preparing the DNA sequence, which encodes for the same peptide, but differs from the native DNA include;

— ease of sequencing or synthesis;

— increased expression of the peptide; and

— preference of certain heterologous hosts for certain codons over others.

These practical considerations are widely known and provide embodiments that may be advantageous to the user of the invention. Thus it is clearly contemplated that the native DNA is not the only embodiment envisioned in this invention.

In addition it is apparent to the skilled artisan that fragments of the protein may be used in screening, drug design and the like, and that the entire peptide may not be required for the purposes of using the invention. Thus it is clearly contemplated that the skilled artisan will understand that the disclosure of the peptide and its uses contemplates the useful peptide fragments.

The practical considerations of protein expression, purification yield, stability, solubility, and the like, are considered by the skilled artisan when choosing whether to use a fragment, and the fragment to be used. As a result, using routine practices in the art, the artisan can, given this disclosure practice the invention using fragments of the protein as well.

The protein or protease itself can be used to determine the binding activity of small molecules to the protein. Drug screening using enzymatic targets is used in the art and can be employed using automated, high throughput technologies.

The inhibition of disintegrin activity may be a predictor of efficacy in the treatment of osteoarthritis, and other diseases involving degeneration of articular cartilage.

Gene therapy

Without being bound by theory it is thought that the metalloprotease is up regulated during osteoarthritis in tissues. We have surprisingly found that a human disintegrin is up-regulated in human chondrocytes during osteoarthritic conditions. Inhibition of signal transduction mechanism is efficacious in disrupting the cascade of events in osteoarthritis and other diseases involving cartilage degeneration. The skilled artisan will recognize that if up-regulation is a cause of the onset of arthritis, then interfering with the activity of this gene may be useful in treating osteoarthritis.

This is done by any of several methods, including gene (i.e., antisense) therapy.

Inhibitors of the disintegrin

The protease of the invention can be used to find inhibitors of the protease. Hence it is useful as a screening tool or for rational drug design. Without being bound by theory, the protease may modulate cellular remodeling and in fact may enhance extracellular matrix remodeling and thus enhance tissue breakdown.. Hence inhibition of disintegrin provides a therapeutic route for treatment of diseases characterized by these processes.

In screening, a drug compound can be used to determine both the quality and quantity of inhibition. As a result such screening provides information for selection of actives, preferably small molecule actives, which are useful in treating these diseases.

In therapy, inhibition of disintegrin metalloprotease activity via binding of small molecular weight, synthetic metalloprotease inhibitors, such as those used to inhibit the matrix metalloproteases would be used to inhibit extracellular matrix remodeling.

Antibodies to the protein

Metalloproteases active at a particularly undesired location (e.g., an organ or certain types of cells) can be targeted by conjugating a metalloprotease inhibitor to a to an antibody or fragment thereof. Conjugation methods are known in the art.

The antibody of the invention can also be conjugated to solid supports. These conjugates can be used as affinity reagents for the purification of a desired metalloprotease, preferably a disintegrin.

In another aspect, the antibody of the invention is directly conjugated to a label. As the antibody binds to the metalloprotease, the label can be used to detect the presence of relatively high levels of metalloprotease in vivo or in vitro cell culture.

In addition, the metalloprotease inhibiting compounds can be conjugated to antibodies. Typical conjugation methods are known in the art. These antibodies are then useful both in therapy and in monitoring the dosage of the inhibitors.

For example, targeting ligand which specifically reacts with a marker for the intended target tissue can be used. Methods for coupling the invention compound to the targeting ligand are well known and are similar to those described below for coupling to carrier. The conjugates are formulated and administered as described above.

Preparation and Use of Antibodies:

Antibodies may be made by several methods, for example, the protein may be injected into suitable (e.g., mammalian) subjects including mice, rabbits, and the like. Preferred protocols involve repeated injection of the immunogen in the presence of adjuvants according to a schedule which boosts production of antibodies in the serum. The titers of the immune serum can readily be measured using immunoassay procedures, now standard in the art.

The antisera obtained can be used directly or monoclonal antibodies may be obtained by harvesting the peripheral blood lymphocytes or the spleen of the immunized animal and immortalizing the antibody-producing cells, followed by identifying the suitable antibody producers using standard immunoassay techniques.

Polyclonal or monoclonal preparations are useful in monitoring therapy or prophylaxis regimens involving the compounds of the invention. Suitable samples such as those derived from blood, serum, urine, or saliva can be tested for the presence of the protein at various times during the treatment protocol using standard immunoassay techniques which employ the antibody preparations of the invention.

These antibodies can also be coupled to labels such as scintigraphic labels, e.g., technetium 99 or I-131, using standard coupling methods. The labeled compounds are administered to subjects to determine the locations of excess amounts of one or more metalloproteases in vivo. Hence a labelled antibody to the protein would operate a a screening tool for such enhanced expression, indicating the disease.

The ability of the antibodies to bind metalloprotease selectively is thus taken advantage of to map the distribution of these enzymes in situ. The techniques can also be employed in histological procedures and the labeled antibodies can be used in competitive immunoassays.

Antibodies are advantageously coupled to other compounds or materials using known methods. For example, materials having a carboxyl functionality, the carboxyl residue can be reduced to an aldehyde and coupled to carrier through reaction with side chain amino groups, optionally followed by reduction of imino linkage formed. The carboxyl residue can also be reacted with side chain amino groups using condensing agents such as dicyclohexyl carbodiimide or other carbodiimide dehydrating agents. Linker compounds can also be used to effect the coupling; both homobifunctional and heterobifunctional linkers are available from Pierce Chemical Company, Rockford, Ill.

These antibodies, when conjugated to a suitable chromatography material are useful in isolating the protein. Separation methods using affinity chromatography are well known in the art, and are within the purview of the skilled artisan.

Disease marker

Without being bound by theory, expression of genes, and preferably this gene may have a restricted tissue distribution and its expression is up regulated by potential osteoarthritis mediators. Enhanced expression of this gene (and hence its protein) for example, in articular chondrocytes provides a marker to monitor the development, including the earliest, asymptomatic stages, and the progression of osteoarthritis. Hence an antibody raised to the protein would operate a a screening tool for such enhanced expression, indicating the disease.

In addition, when used in a disease screen, antibodies can be conjugated to chromophore or fluorophore containing materials, or can be conjugated to enzymes which produce chromophores or fluorophores in certain conditions. These conjugating materials and methods are well known in the art. When used in this manner detection of the protein by immunoassay is straightforward to the skilled artisan. Body fluids, for example can be screened in this manner for calibration, and detection of distribution of metalloproteases, or increased levels of these proteases.

When used in this way the invention is a useful diagnostic and/or clinical marker for metalloprotease mediated diseases, such as osteoarthritis or other articular cartilage degenerative diseases. When disease is detected, it may be treated before the onset of symptom or debilitation.

Furthermore, such antibodies can be used to target diseased tissue, for detection or treatment as described above.

EXAMPLES

The following non-limiting examples illustrate a preferred embodiment of the present invention, and briefly describe the uses of the present invention. These

examples are provided for the guidance of the skilled artisan, and do not limit the invention in any way. Armed with this disclosure and these examples the skilled artisan is capable of making and using the claimed invention.

Standard starting materials are used for these examples. Many of these materials are known and commercially available. For example E. coli CJ236 and JM101 are known strains, pUB110 is a known plasmid and Kunkel method mutagenesis is also well known in the art.

Variants may be made by expression systems and by various methods in various hosts, these methods are within the scope of the practice of the skilled artisan in molecular biology, biochemistry or other arts related to biotechnology.

Example 1

RNA was isolated from unstimulated and interleukin-1 stimulated cultures of normal human articular chondrocytes. The RNA was reverse transcribed into cDNA. The cDNA was subjected to a modified differential display procedure using a series of random primers.

PCR samples generated from both stimulated and unstimulated chondrocytes were electrophoreses in adjacent lanes on polyacrylamide gels. The differentially expressed band was excised from the gel, cloned, and sequenced. The differential expression of the gene was confirmed by RNase protection and nuclear run on experiments.

Example 2

A novel partial human cDNA coding the protein is cloned from primary cultures f interleukin-1 stimulated human articular (femoral head) chondrocytes, using known methods.

The same sequence is found, and the gene completed by screening of human cDNA libraries to obtain full length clones.

Example 3

The cloned DNA of example 2 was placed in pUB110 using known methods.

This plasmid is used to transform *E. coli* and provides a template for site-directed mutagenesis to create new mutants. Kunkel method mutagenesis performed altering GLN 1 ALA.

Example 4

[^{125}I] disintegrin antibody is prepared using IODOBEADS (Pierce, Rockford, IL; immobilized chloramine-T on nonporous polystyrene beads). Lyophilized antibody (2 µg) is taken up in 50 µl of 10 mM acetic acid and added to 450 µl of phosphate-buffered saline (PBS) (Sigma, St. Louis, MO) on ice. To the tube is added 500 µCurie of ^{125}I

(Amersham, Arlington Heights, IL) (2200Ci/mmol) in 5 μl, and one IODOBEAD. The reaction is incubated on ice for 10 min with occasional shaking. The reaction is then terminated by removal of the reaction from the IODOBEAD. To remove unreacted ^{125}I, the mixture is applied to a PD-10 gel filtration column.

Example 5

A fluorogenic peptide (Bachem, Guelph Mills, King of Prussia, Pa) is mixed with the disintegrin and change in the fluorecence is evaluated at 2 min, as a control.. Then the fluorogenic peptide is mixed with the disintegrin in the presence of the compound in evaluation in a separate run, with evaluation at 2 minutes. Data are evaluated using standard methodology to provide relative binding of the evaluated compound.

Example 6

0.5ml of synovial fluid from the left knee of a patient is withdrawn and tested for elevated levels disintegrin by ELISA. The results indicate higher than normal disintegrin level. The patient is prescribed a prophylactic dose of a disintegrin inhibitor, and is administered an injection of same in the left knee before leaving the clinician's office.

Example 7

Inhibition of extracellular matrix remodeling is explored via inhibition of disintegrin metalloprotease activity. Using a small molecular weight, synthetic metalloprotease inhibitor, such as those used to inhibit the matrix metalloproteases, tissue integrety and proteoglycan is monitored.

A sample of mouse derived articular cartilage is grown in a 1 micromolar solution of a small molecular weight disintegrin inhibitor. The experiment is controlled and compared to an identical culture grown with no inhibitor.

The assay of the culture after 7 days shows that the inhibited culture has less tissue breakdown and less proteoglycan present in the serum of the culture. The result is consistant with the inhibited aggrecanase activity. Inhibition of aggrecanase would inhibit tissue breakdown and reduce the release of proteoglycan.

Example 8

Inhibition of proteolytic processing resulting in the release from the membrane bound form of the disintegrin metalloprotease domain inhibits "second messenger" signaling of the membrane bound disintegrin molecule. Such second messenger signaling would result in cellular phenotypic changes, changes in gene expression, changes in mitotic activity, and the like.

Cells known to contain disintegrin are treated with a serine protease. Proteins released from the cell are measured by standard methods. Specifically the

metalloprotease activity is monitored via literature methods. The amount of metalloprotease released is correlated to the amount of serine protease used to treat the cells.

Increaeses, versus control, in src tyrosine kinase activity are measured by Western blot analysis of intracellular proteins using monoclonal antibodies specific for phosphotyrosine following cleavage and release of the disintegrin metalloprotease. Controls are cells that have not been treated with serine protease.

src tyrosine kinase activity in the cell (or is it cell culture) is measured by literature meathods. Release of the metalloprotease domain of the disintegrin is also monitored via literature methods. There is a direct correlation between release of the metalloprotease domain and increases in intracellular src tyrosine kinase activity. This result is consistent with stimulation of disintegrin-mediated cell signalling by stimulation of the src tyrosine kinase cascade.

Example 9

Inhibition of intercellular adhesion molecules, or extracellular matrix components results in the inhibition of phenotypic changes, including changes in cell shape, associated with such interactions, as described in Example 8.

Integrin binding is measured with a peptide containing the sequence RGD, using the protocol of Example 8. Integrin binding is measured via competitive assay, using cellular changes in shape visible via microscopy. The peptide inhibits the cellular changes as in Example 8.

This result is consistent with competition with or blocking of the interaction of disintegrin. The RGD peptide inhibits cellular changes in chondrocytes. The osteoarthritis phenotype, characterized by increased matrix synthesis and accelerated matrix metalloprotease activity does not occur. Other readily assayable cellular changes can be used to moniter this result, including gene expression, changes in mitotic activity, and the like.

Example 10

A small molecular weight metalloprotease inhibitor is used to treat a tissue culture according to the method of Example 7. The release of TNF-α from the cell membrane is measured by literature methods. The inhibitor of Example 7 also decreases the amount of TNF-α secreted from the cell membrane.

This is consistant with the theory that inhibition of disintegrin metalloprotease activity will result in the inhibition of a disintegrin associated inflammation cascade and

secretase activity. It is contemplated that monitoring the release of cytokines or IL-1 from the cell membrane, and the like will produce the same result.

All references described herein are hereby incorporated by reference.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: TINDAL, MICHAEL H.
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(ii) TITLE OF INVENTION: USE OF A NOVEL DISINTEGRIN METALLOPROTEASE, ITS MUTANTS, FRAGMENTS AND THE LIKE

(iii) NUMBER OF SEQUENCES: 4

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(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 37,343
(C) REFERENCE/DOCKET NUMBER: 5980

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(B) TELEFAX: 513/622-0270

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1961 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..1474

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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        210                 215                 220

GAT GAA TGC TGC TTC GAT GCA AAT CAA CCA GAG GGA AGA AAA TGC AAA        718
Asp Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg Lys Cys Lys
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    225                 230                 235

CTG AAA CCT GGG AAA CAG TGC AGT CCA AGT CAA GGT CCT TGT TGT ACA      766
Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr
240                 245                 250                 255

GCA CAG TGT GCA TTC AAG TCA AAG TCT GAG AAG TGT CGG GAT GAT TCA      814
Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser
                260                 265                 270

GAC TGT GCA AGG GAA GGA ATA TGT AAT GGC TTC ACA GCT CTC TGC CCA      862
Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro
        275                 280                 285

GCA TCT GAC CCT AAA CCA AAC TTC ACA GAC TGT AAT AGG CAT ACA CAA      910
Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln
    290                 295                 300

GTG TGC ATT AAT GGG CAA TGT GCA GGT TCT ATC TGT GAG AAA TAT GGC      958
Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly
305                 310                 315

TTA GAG GAG TGT ACG TGT GCC AGT TCT GAT GGC AAA GAT GAT AAA GAA      1006
Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu
320                 325                 330                 335

TTA TGC CAT GTA TGC TGT ATG AAG AAA ATG GAC CCA TCA ACT TGT GCC      1054
Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala
                340                 345                 350

AGT ACA GGG TCT GTG CAG TGG AGT AGG CAC TTC AGT GGT CGA ACC ATC      1102
Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile
            355                 360                 365

ACC CTG CAA CCT GGA TCC CCT TGC AAC GAT TTT AGA GGT TAC TGT GAT      1150
Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp
        370                 375                 380

GTT TTC ATG CGG TGC AGA TTA GTA GAT GCT GAT GGT CCT CTA GCT AGG      1198
Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg
    385                 390                 395

CTT AAA AAA GCA ATT TTT AGT CCA GAG CTC TAT GAA AAC ATT GCT GAA      1246
Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu
400                 405                 410                 415

TGG ATT GTG GCT CAT TGG TGG GCA GTA TTA CTT ATG GGA ATT GCT CTG      1294
Trp Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly Ile Ala Leu
                420                 425                 430

ATC ATG CTA ATG GCT GGA TTT ATT AAG ATA TGC AGT GTT CAT ACT CCA      1342
Ile Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val His Thr Pro
            435                 440                 445

AGT AGT AAT CCA AAG TTG CCT CCT CCT AAA CCA CTT CCA GGC ACT TTA      1390
Ser Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro Gly Thr Leu
        450                 455                 460

AAG AGG AGG AGA CCT CCA CAG CCC ATT CAG CAA CCC CAG CGT CAG CGG      1438
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Lys Arg Arg Arg Pro Pro Gln Pro Ile Gln Gln Pro Gln Arg Gln Arg
    465                 470                 475

CCC CGA GAG AGT TAT CAA ATG GGA CAC ATG AGA CGC TAACTGCAGC          1484
Pro Arg Glu Ser Tyr Gln Met Gly His Met Arg Arg
480                 485                 490

TTTTGCCTTG GTTCTTCCTA GTGCCTACAA TGGGAAAACT TCACTCCAAA GAGAAACCTA  1544

TTAAGTCATC ATCTCCAAAC TAAACCCTCA CAAGTAACAG TTGAAGAAAA AATGGCAAGA  1604

GATCATATCC TCAGACCAGG TGGAATTACT TAAATTTTAA AGCCTGAAAA TTCCAATTTG  1664

GGGGTGGGAG GTGGAAAAGG AACCCAATTT TCTTATGAAC AGATATTTTT AACTTAATGG  1724

CACAAAGTCT TAGAATATTA TTATGTGCCC CGTGTTCCCT GTTCTTCGTT GCTGCATTTT  1784

CTTCACTTGC AGGCAAACTT GGCTCTCAAT AAACTTTTCG GTCCAGACCA CAGACTTCTC  1844

CGGAATCCGT AACATCAGTT TCATGGTGAA ACGCATAAGA ATCAATACAA CTGCTGATGA  1904

GAAGGACCCT ACAAATCCTT TCCGTTTCCC AAATATTAGT GTGGAGAAGT TAAACAA     1961
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Gln Thr Thr Asp Phe Ser Gly Ile Arg Asn Ile Ser Phe Met Val Lys
 1               5                  10                  15

Arg Ile Arg Ile Asn Thr Thr Ala Asp Glu Lys Asp Pro Thr Asn Pro
            20                  25                  30

Phe Arg Phe Pro Asn Ile Ser Val Glu Lys Phe Leu Glu Leu Asn Ser
        35                  40                  45

Glu Gln Asn His Asp Asp Tyr Cys Leu Ala Tyr Val Phe Thr Asp Arg
    50                  55                  60

Asp Phe Asp Asp Gly Val Leu Gly Leu Ala Trp Val Gly Ala Pro Ser
65                  70                  75                  80

Gly Ser Ser Gly Gly Ile Cys Glu Lys Ser Lys Leu Tyr Ser Asp Gly
                85                  90                  95

Lys Lys Lys Ser Leu Asn Thr Gly Ile Ile Thr Val Gln Asn Tyr Gly
            100                 105                 110

Ser His Val Pro Pro Lys Val Ser His Ile Thr Phe Ala His Glu Val
        115                 120                 125
```

Gly His Asn Phe Gly Ser Pro His Asp Ser Gly Thr Glu Cys Thr Pro
130 135 140

Gly Glu Ser Lys Asn Leu Gly Gln Lys Glu Asn Gly Asn Tyr Ile Met
145 150 155 160

Tyr Ala Arg Ala Thr Ser Gly Asp Lys Leu Asn Asn Asn Lys Phe Ser
165 170 175

Leu Cys Ser Ile Arg Asn Ile Ser Gln Val Leu Glu Lys Lys Arg Asn
180 185 190

Asn Cys Phe Val Glu Ser Gly Gln Pro Ile Cys Gly Asn Gly Met Val
195 200 205

Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp Gln Cys Lys Asp
210 215 220

Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg Lys Cys Lys Leu
225 230 235 240

Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr Ala
245 250 255

Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser Asp
260 265 270

Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro Ala
275 280 285

Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln Val
290 295 300

Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly Leu
305 310 315 320

Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu Leu
325 330 335

Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala Ser
340 345 350

Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile Thr
355 360 365

Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp Val
370 375 380

Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg Leu
385 390 395 400

Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu Trp
405 410 415

Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly Ile Ala Leu Ile
420 425 430

Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val His Thr Pro Ser
435 440 445

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Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro Gly Thr Leu Lys
    450                 455                 460

Arg Arg Arg Pro Pro Gln Pro Ile Gln Gln Pro Gln Arg Gln Arg Pro
465                 470                 475                 480

Arg Glu Ser Tyr Gln Met Gly His Met Arg Arg
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2763 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17..2414

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGCGGCGGCA CGGAAG ATG GTG TTG CTG AGA GTG TTA ATT CTG CTC CTC          49
                  Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu
                                  495                 500

TCC TGG GCG GCG GGG ATG GGA GGT CAG TAT GGG AAT CCT TTA AAT AAA        97
Ser Trp Ala Ala Gly Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys
        505                 510                 515

TAT ATC AGA CAT TAT GAA GGA TTA TCT TAC AAT GTG GAT TCA TTA CAC       145
Tyr Ile Arg His Tyr Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His
    520                 525                 530

CAA AAA CAC CAG CGT GCC AAA AGA GCA GTC TCA CAT GAA GAC CAA TTT       193
Gln Lys His Gln Arg Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe
535                 540                 545                 550

TTA CGT CTA GAT TTC CAT GCC CAT GGA AGA CAT TTC AAC CTA CGA ATG       241
Leu Arg Leu Asp Phe His Ala His Gly Arg His Phe Asn Leu Arg Met
                555                 560                 565

AAG AGG GAC ACT TCC CTT TTC AGT GAT GAA TTT AAA GTA GAA ACA TCA       289
Lys Arg Asp Thr Ser Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser
            570                 575                 580

AAT AAA GTA CTT GAT TAT GAT ACC TCT CAT ATT TAC ACT GGA CAT ATT       337
Asn Lys Val Leu Asp Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile
        585                 590                 595

TAT GGT GAA GAA GGA AGT TTT AGC CAT GGG TCT GTT ATT GAT GGA AGA       385
Tyr Gly Glu Glu Gly Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg
    600                 605                 610
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TTT GAA GGA TTC ATC CAG ACT CGT GGT GGC ACA TTT TAT GTT GAG CCA      433
Phe Glu Gly Phe Ile Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro
615                 620                 625                 630

GCA GAG AGA TAT ATT AAA GAC CGA ACT CTG CCA TTT CAC TCT GTC ATT      481
Ala Glu Arg Tyr Ile Lys Asp Arg Thr Leu Pro Phe His Ser Val Ile
                635                 640                 645

TAT CAT GAA GAT GAT ATT AGT GAA AGG CTT AAA CTG AGG CTT AGA AAA      529
Tyr His Glu Asp Asp Ile Ser Glu Arg Leu Lys Leu Arg Leu Arg Lys
            650                 655                 660

CTT ATG TCA CTT GAG TTG TGG ACC TCC TGT TGT TTA CCC TGT GCT CTT      577
Leu Met Ser Leu Glu Leu Trp Thr Ser Cys Cys Leu Pro Cys Ala Leu
        665                 670                 675

CTG CTT CAC TCA TGG AAG AAA GCT GTA AAT TCT CAC TGC CTT TAC TTC      625
Leu Leu His Ser Trp Lys Lys Ala Val Asn Ser His Cys Leu Tyr Phe
    680                 685                 690

AAG GAT TTC TGG GGC TTT TCT GAA ATC TAC TAT CCC CAT AAA TAC GGT      673
Lys Asp Phe Trp Gly Phe Ser Glu Ile Tyr Tyr Pro His Lys Tyr Gly
695                 700                 705                 710

CCT CAG GGC GGC TGT GCA GAT CAT TCA GTA TTT GAA AGA ATG AGG AAA      721
Pro Gln Gly Gly Cys Ala Asp His Ser Val Phe Glu Arg Met Arg Lys
                715                 720                 725

TAC CAG ATG ACT GGT GTA GAG GAA GTA ACA CAG ATA CCT CAA GAA GAA      769
Tyr Gln Met Thr Gly Val Glu Glu Val Thr Gln Ile Pro Gln Glu Glu
            730                 735                 740

CAT GCT GCT AAT GGT CCA GAA CTT CTG AGG AAA AGA CGT ACA ACT TCA      817
His Ala Ala Asn Gly Pro Glu Leu Leu Arg Lys Arg Arg Thr Thr Ser
        745                 750                 755

GCT GAA AAA AAT ACT TGT CAG CTT TAT ATT CAG ACT GAT CAT TTG TTC      865
Ala Glu Lys Asn Thr Cys Gln Leu Tyr Ile Gln Thr Asp His Leu Phe
    760                 765                 770

TTT AAA TAT TAC GGA ACA CGA GAA GCT GTG ATT GCC CAG ATA TCC AGT      913
Phe Lys Tyr Tyr Gly Thr Arg Glu Ala Val Ile Ala Gln Ile Ser Ser
775                 780                 785                 790

CAT GTT AAA GCG ATT GAT ACA ATT TAC CAG ACC ACA GAC TTC TCC GGA      961
His Val Lys Ala Ile Asp Thr Ile Tyr Gln Thr Thr Asp Phe Ser Gly
                795                 800                 805

ATC CGT AAC ATC AGT TTC ATG GTG AAA CGC ATA AGA ATC AAT ACA ACT     1009
Ile Arg Asn Ile Ser Phe Met Val Lys Arg Ile Arg Ile Asn Thr Thr
            810                 815                 820

GCT GAT GAG AAG GAC CCT ACA AAT CCT TTC CGT TTC CCA AAT ATT AGT     1057
Ala Asp Glu Lys Asp Pro Thr Asn Pro Phe Arg Phe Pro Asn Ile Ser
        825                 830                 835

GTG GAG AAG TTT CTG GAA TTG AAT TCT GAG CAG AAT CAT GAT GAC TAC     1105
Val Glu Lys Phe Leu Glu Leu Asn Ser Glu Gln Asn His Asp Asp Tyr
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    840                 845                 850

TGT TTG GCC TAT GTC TTC ACA GAC CGA GAT TTT GAT GAT GGC GTA CTT      1153
Cys Leu Ala Tyr Val Phe Thr Asp Arg Asp Phe Asp Asp Gly Val Leu
855                 860                 865                 870

GGT CTG GCT TGG GTT GGA GCA CCT TCA GGA AGC TCT GGA GGA ATA TGT      1201
Gly Leu Ala Trp Val Gly Ala Pro Ser Gly Ser Ser Gly Gly Ile Cys
                875                 880                 885

GAA AAA AGT AAA CTC TAT TCA GAT GGT AAG AAG AAG TCC TTA AAC ACT      1249
Glu Lys Ser Lys Leu Tyr Ser Asp Gly Lys Lys Lys Ser Leu Asn Thr
            890                 895                 900

GGA ATT ATT ACT GTT CAG AAC TAT GGG TCT CAT GTA CCT CCC AAA GTC      1297
Gly Ile Ile Thr Val Gln Asn Tyr Gly Ser His Val Pro Pro Lys Val
        905                 910                 915

TCT CAC ATT ACT TTT GCT CAC GAA GTT GGA CAT AAC TTT GGA TCC CCA      1345
Ser His Ile Thr Phe Ala His Glu Val Gly His Asn Phe Gly Ser Pro
    920                 925                 930

CAT GAT TCT GGA ACA GAG TGC ACA CCA GGA GAA TCT AAG AAT TTG GGT      1393
His Asp Ser Gly Thr Glu Cys Thr Pro Gly Glu Ser Lys Asn Leu Gly
935                 940                 945                 950

CAA AAA GAA AAT GGC AAT TAC ATC ATG TAT GCA AGA GCA ACA TCT GGG      1441
Gln Lys Glu Asn Gly Asn Tyr Ile Met Tyr Ala Arg Ala Thr Ser Gly
                955                 960                 965

GAC AAA CTT AAC AAC AAT AAA TTC TCA CTC TGT AGT ATT AGA AAT ATA      1489
Asp Lys Leu Asn Asn Asn Lys Phe Ser Leu Cys Ser Ile Arg Asn Ile
            970                 975                 980

AGC CAA GTT CTT GAG AAG AAG AGA AAC AAC TGT TTT GTT GAA TCT GGC      1537
Ser Gln Val Leu Glu Lys Lys Arg Asn Asn Cys Phe Val Glu Ser Gly
        985                 990                 995

CAA CCT ATT TGT GGA AAT GGA ATG GTA GAA CAA GGT GAA GAA TGT GAT      1585
Gln Pro Ile Cys Gly Asn Gly Met Val Glu Gln Gly Glu Glu Cys Asp
    1000                1005                1010

TGT GGC TAT AGT GAC CAG TGT AAA GAT GAA TGC TGC TTC GAT GCA AAT      1633
Cys Gly Tyr Ser Asp Gln Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn
1015                1020                1025                1030

CAA CCA GAG GGA AGA AAA TGC AAA CTG AAA CCT GGG AAA CAG TGC AGT      1681
Gln Pro Glu Gly Arg Lys Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser
                1035                1040                1045

CCA AGT CAA GGT CCT TGT TGT ACA GCA CAG TGT GCA TTC AAG TCA AAG      1729
Pro Ser Gln Gly Pro Cys Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys
            1050                1055                1060

TCT GAG AAG TGT CGG GAT GAT TCA GAC TGT GCA AGG GAA GGA ATA TGT      1777
Ser Glu Lys Cys Arg Asp Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys
        1065                1070                1075

AAT GGC TTC ACA GCT CTC TGC CCA GCA TCT GAC CCT AAA CCA AAC TTC      1825
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Asn Gly Phe Thr Ala Leu Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe
    1080                1085                1090

ACA GAC TGT AAT AGG CAT ACA CAA GTG TGC ATT AAT GGG CAA TGT GCA      1873
Thr Asp Cys Asn Arg His Thr Gln Val Cys Ile Asn Gly Gln Cys Ala
1095                1100                1105                1110

GGT TCT ATC TGT GAG AAA TAT GGC TTA GAG GAG TGT ACG TGT GCC AGT      1921
Gly Ser Ile Cys Glu Lys Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser
                1115                1120                1125

TCT GAT GGC AAA GAT GAT AAA GAA TTA TGC CAT GTA TGC TGT ATG AAG      1969
Ser Asp Gly Lys Asp Asp Lys Glu Leu Cys His Val Cys Cys Met Lys
            1130                1135                1140

AAA ATG GAC CCA TCA ACT TGT GCC AGT ACA GGG TCT GTG CAG TGG AGT      2017
Lys Met Asp Pro Ser Thr Cys Ala Ser Thr Gly Ser Val Gln Trp Ser
        1145                1150                1155

AGG CAC TTC AGT GGT CGA ACC ATC ACC CTG CAA CCT GGA TCC CCT TGC      2065
Arg His Phe Ser Gly Arg Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys
    1160                1165                1170

AAC GAT TTT AGA GGT TAC TGT GAT GTT TTC ATG CGG TGC AGA TTA GTA      2113
Asn Asp Phe Arg Gly Tyr Cys Asp Val Phe Met Arg Cys Arg Leu Val
1175                1180                1185                1190

GAT GCT GAT GGT CCT CTA GCT AGG CTT AAA AAA GCA ATT TTT AGT CCA      2161
Asp Ala Asp Gly Pro Leu Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro
                1195                1200                1205

GAG CTC TAT GAA AAC ATT GCT GAA TGG ATT GTG GCT CAT TGG TGG GCA      2209
Glu Leu Tyr Glu Asn Ile Ala Glu Trp Ile Val Ala His Trp Trp Ala
            1210                1215                1220

GTA TTA CTT ATG GGA ATT GCT CTG ATC ATG CTA ATG GCT GGA TTT ATT      2257
Val Leu Leu Met Gly Ile Ala Leu Ile Met Leu Met Ala Gly Phe Ile
        1225                1230                1235

AAG ATA TGC AGT GTT CAT ACT CCA AGT AGT AAT CCA AAG TTG CCT CCT      2305
Lys Ile Cys Ser Val His Thr Pro Ser Ser Asn Pro Lys Leu Pro Pro
    1240                1245                1250

CCT AAA CCA CTT CCA GGC ACT TTA AAG AGG AGG AGA CCT CCA CAG CCC      2353
Pro Lys Pro Leu Pro Gly Thr Leu Lys Arg Arg Arg Pro Pro Gln Pro
1255                1260                1265                1270

ATT CAG CAA CCC CAG CGT CAG CGG CCC CGA GAG AGT TAT CAA ATG GGA      2401
Ile Gln Gln Pro Gln Arg Gln Arg Pro Arg Glu Ser Tyr Gln Met Gly
                1275                1280                1285

CAC ATG AGA CGC  T AACTGCAGCT TTTGCCTTGG TTCTTCCTAG TGCCTACAAT      2454
His Met Arg Arg
            1290

GGGAAAACTT CACTCCAAAG AGAAACCTAT TAAGTCATCA TCTCCAAACT AAACCCTCAC    2514

AAGTAACAGT TGAAGAAAAA ATGGCAAGAG ATCATATCCT CAGACCAGGT GGAATTACTT    2574
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AAATTTTAAA GCCTGAAAAT TCCAATTTGG GGGTGGGAGG TGGAAAAGGA ACCCAATTTT 2634
CTTATGAACA GATATTTTTA ACTTAATGGC ACAAAGTCTT AGAATATTAT TATGTGCCCC 2694
GTGTTCCCTG TTCTTCGTTG CTGCATTTTC TTCACTTGCA GGCAAACTTG GCTCTCAATA 2754
AACTTTTCG 2763

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 799 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu Ser Trp Ala Ala Gly
1 5 10 15

Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys Tyr Ile Arg His Tyr
20 25 30

Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His Gln Lys His Gln Arg
35 40 45

Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe Leu Arg Leu Asp Phe
50 55 60

His Ala His Gly Arg His Phe Asn Leu Arg Met Lys Arg Asp Thr Ser
65 70 75 80

Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser Asn Lys Val Leu Asp
85 90 95

Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile Tyr Gly Glu Glu Gly
100 105 110

Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg Phe Glu Gly Phe Ile
115 120 125

Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro Ala Glu Arg Tyr Ile
130 135 140

Lys Asp Arg Thr Leu Pro Phe His Ser Val Ile Tyr His Glu Asp Asp
145 150 155 160

Ile Ser Glu Arg Leu Lys Leu Arg Leu Arg Lys Leu Met Ser Leu Glu
165 170 175

Leu Trp Thr Ser Cys Cys Leu Pro Cys Ala Leu Leu Leu His Ser Trp
180 185 190

Lys Lys Ala Val Asn Ser His Cys Leu Tyr Phe Lys Asp Phe Trp Gly
195 200 205

21

AAATTTTAAA GCCTGAAAAT TCCAATTTGG GGGTGGGAGG TGGAAAAGGA ACCCAATTTT 2634
CTTATGAACA GATATTTTTA ACTTAATGGC ACAAAGTCTT AGAATATTAT TATGTGCCCC 2694
GTGTTCCCTG TTCTTCGTTG CTGCATTTTC TTCACTTGCA GGCAAACTTG GCTCTCAATA 2754
AACTTTTCG 2763

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 799 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu Ser Trp Ala Ala Gly
1 5 10 15

Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys Tyr Ile Arg His Tyr
20 25 30

Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His Gln Lys His Gln Arg
35 40 45

Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe Leu Arg Leu Asp Phe
50 55 60

His Ala His Gly Arg His Phe Asn Leu Arg Met Lys Arg Asp Thr Ser
65 70 75 80

Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser Asn Lys Val Leu Asp
85 90 95

Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile Tyr Gly Glu Glu Gly
100 105 110

Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg Phe Glu Gly Phe Ile
115 120 125

Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro Ala Glu Arg Tyr Ile
130 135 140

Lys Asp Arg Thr Leu Pro Phe [illegible]

Phe Ser Glu Ile Tyr Tyr Pro His Lys Tyr Gly Pro Gln Gly Gly Cys
210 215 220

Ala Asp His Ser Val Phe Glu Arg Met Arg Lys Tyr Gln Met Thr Gly
225 230 235 240

Val Glu Glu Val Thr Gln Ile Pro Gln Glu Glu His Ala Ala Asn Gly
245 250 255

Pro Glu Leu Leu Arg Lys Arg Arg Thr Thr Ser Ala Glu Lys Asn Thr
260 265 270

Cys Gln Leu Tyr Ile Gln Thr Asp His Leu Phe Phe Lys Tyr Tyr Gly
275 280 285

Thr Arg Glu Ala Val Ile Ala Gln Ile Ser Ser His Val Lys Ala Ile
290 295 300

Asp Thr Ile Tyr Gln Thr Thr Asp Phe Ser Gly Ile Arg Asn Ile Ser
305 310 315 320

Phe Met Val Lys Arg Ile Arg Ile Asn Thr Thr Ala Asp Glu Lys Asp
325 330 335

Pro Thr Asn Pro Phe Arg Phe Pro Asn Ile Ser Val Glu Lys Phe Leu
340 345 350

Glu Leu Asn Ser Glu Gln Asn His Asp Asp Tyr Cys Leu Ala Tyr Val
355 360 365

Phe Thr Asp Arg Asp Phe Asp Asp Gly Val Leu Gly Leu Ala Trp Val
370 375 380

Gly Ala Pro Ser Gly Ser Ser Gly Gly Ile Cys Glu Lys Ser Lys Leu
385 390 395 400

Tyr Ser Asp Gly Lys Lys Lys Ser Leu Asn Thr Gly Ile Ile Thr Val
405 410 415

Gln Asn Tyr Gly Ser His Val Pro Pro Lys Val Ser His Ile Thr Phe
420 425 430

Ala His Glu Val Gly His Asn Phe Gly Ser Pro His Asp Ser Gly Thr
435 440 445

Glu Cys Thr Pro Gly Glu Ser Lys Asn Leu Gly Gln Lys Glu Asn Gly
450 455 460

Asn Tyr Ile Met Tyr Ala Arg Ala Thr Ser Gly Asp Lys Leu Asn Asn
465 470 475 480

Asn Lys Phe Ser Leu Cys Ser Ile Arg Asn Ile Ser Gln Val Leu Glu
485 490 495

Lys Lys Arg Asn Asn Cys Phe Val Glu Ser Gly Gln Pro Ile Cys Gly
500 505 510

Asn Gly Met Val Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp
515 520 525

Gln Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg
530 535 540

Lys Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro
545 550 555 560

Cys Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg
565 570 575

Asp Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala
580 585 590

Leu Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg
595 600 605

His Thr Gln Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu
610 615 620

Lys Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp
625 630 635 640

Asp Lys Glu Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser
645 650 655

Thr Cys Ala Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly
660 665 670

Arg Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly
675 680 685

Tyr Cys Asp Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro
690 695 700

Leu Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn
705 710 715 720

Ile Ala Glu Trp Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly
725 730 735

Ile Ala Leu Ile Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val
740 745 750

His Thr Pro Ser Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro
755 760 765

Gly Thr Leu Lys Arg Arg Arg Pro Pro Gln Pro Ile Gln Gln Pro Gln
770 775 780

Arg Gln Arg Pro Arg Glu Ser Tyr Gln Met Gly His Met Arg Arg
785 790 795

WHAT IS CLAIMED IS:

1. A DNA fragment encoding a human disintegrin expressed differentially during arthritis development, capable as being used as a screen disintegrin antagonism, drug design and screening.

2. A human disintegrin according to Claim 1 of a molecular weight, and solubility useful as a drug screening agent.

3. A human disintegrin according to Claim 1 in essentially pure form.

4. A screening method for compounds capable of binding to a human disintegrin, comprising the disintegrin of Claim 1.

5. A screening kit for compounds capable of binding to a human disintegrin, comprising the disintegrin of Claim 1.

6. An antibody, or fragment thereof, to human disintegrin of Claim 1.

7. A screening method for a metalloprotease mediated disease comprising the administration of an antibody according to Claim 6 and observing its effect.

8. A screening method for osteoarthritis comprising the administration of an antibody according to Claim 7 and observing its effect.

9. A screening method for osteoarthritis according to Claim 7, where blood, synovial fluid or other body fluids are screened.

10. A screening kit for osteoarthritis comprising an antibody, or fragment thereof, to human disintegrin of Claim 6.

11. A screening method, according to Claim 4, useful in determining relative potency in treating osteoarthritis.

12. DNA encoding the disintegrin of Claim 1 (Seq ID NO 2).

13. An expression vector or plasmid comprising the DNA of Claim 12.

14. A cell comprising the DNA of Claim 12.

15. A cell comprising the expression vector or plasmid of Claim 13.

16. The cell of Claim 14 where the DNA is foreign to that cell.

17. An inhibitor of the human disintegrin of Claim 2.

18. A method of treating a disease state associated with disintegrin activity.

19. The disintegrin of Claim 2, wherein the disintegrin is aggrocanase.

20. A method of treating a disease state according to Claim 18 wherein the disease is an arthropothy.

21. A method according to Claim 20, wherein the disease is osteoarthritis.

22. The disintegrin of Claim 2, wherein the disintegrin modulates tissue remodeling or breakdown.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03217

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :536/23.5; 435/7.1, 320.1, 325; 424/130.1; 514/2; 530/350;
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.1; 435/7.1, 4, 320.1, 325; 424/130.1; 514/2; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genbank ACC. No. D31872, KATAGIRI et al., 'Human metalloprotease/disintegrin-like (MDC) gene:exon-intron organization and alternative splicing,' sequence, Cytogenet. Cell Genet., 68 (1-2), 39-44, 1995, first submitted 18 June 1994, see entire sequence listing.	1-22
X	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genebank ACC. No. U41767, BLOBEL, C. P., DIRECT SUBMISSION, sequence submitted 04 December 1995, see entire sequence listing.	1
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Y		2-5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 APRIL 1997	Date of mailing of the international search report 09 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DEBORAH J. R. CLARK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03217

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genebank ACC. No. U41766, BLOBEL C. P., DIRECT SUBMISSION, sequence submitted 04 December 1995, see entire sequence listing.	1 ----- 2-5
X, P --- Y, P	KRATZSCHMAR et al. Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD Integrin binding sequence. J. of Biol. Chem. 01 March 1996, Vol. 271, No. 9, pages 4593-4596, especially pages 4593-4594.	1, 4, 6 ----- 2, 3, 5, 7
X --- Y	WESKAMP et al. MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. J. of Cell Biol. 04 February 1996, Vol. 132, No. 4, pages 717-726, especially pages 717-718.	1, 4, 6 ----- 2, 3, 5, 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03217

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 21/02; G01N 33/53; C12N 15/63, 5/16; A61K 39/395, 38/00; C07K 14/435

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Medline, Scisearch, Biosis, Embase, CAPlus, WPIDS, Genbank
Search terms: disintegrin, DNA, cDNA, coding sequence, screen, bind, metalloprotease, osteoarthritis, aggrocanase, arthritis, human, Haqqi, T/au, Tindal, M/au